

IN THE SPECIFICATION

On page 6 please insert after the paragraph “The principles of LLLME will be explained in more detail below” insert as a paragraph:

The three liquids (LLL) in liquid-liquid-liquid micro extraction (LLLME) are set forth in Figures 3 and 4. The first liquid is the sample solution, the second liquid is the liquid membrane and the third liquid is the acceptor solution. Liquid-liquid micro extraction (LLME) on the other hand utilized non-liquid membrane as shown in Figure 2.

On page 11, please replace the last paragraph with the following paragraph:

In LSLME the membrane can be used as it is. In LLLME the liquid membrane is formed by dipping ~~the~~^a hollow fibre into the organic solvent for 5-30 sec. to allow the solvent to penetrate into the pores of the fibre. Acceptor solution is then filled into the fibre by a syringe. Normally, fibres with an inner tube volume of 10 μ l are preferred, since 10 μ l of acceptor solution gives high analyte enrichments and 10 μ l volumes can be handled with commercially available syringes. The acceptor solution has a pH where the target analytes are charged. Extraction is performed by connecting the LLLME device to the sample vial. The sample filled into the sample vial is buffered to a pH where the analytes are neutral. Typical sample volumes are 0.5-5 ml of a biological fluid. An internal standard is always added to the sample solution before extraction to compensate for fluctuations in the procedure. Extraction is performed by stirring, for example with a magnetic stir bar placed in the sample vial. ~~Extraction is continued until equilibrium between the three phases is established.~~ When equilibrium is reached (15-60 min) the acceptor solution is collected with a syringe and filled into autosampler vials for automated injection into the analytical instrument.

On page 12 please replace the third paragraph with the following paragraph:

LLME is demonstrated by the extraction of 5 nmol/ml sample solutions of diazepam (D) and prazepam (P) prepared in 1.0 M acetate buffer pH 5.5, in urine and in human plasma. A standard solution in octanol (5 nmol/ml) was prepared as a reference solution for direct injection into the gas

chromatograph. The pH of the standard solution in urine was adjusted to pH 5.5 before extraction. To an aliquote of plasma (1080 μ l) was added 120 μ l methanol to reduce the protein binding of the benzodiazepines prior to extraction and the mixture was agitated for 1 min. LLME was accomplished by placing 1.2 ml of the sample solutions in 2 ml autosampler vials (Chromacol, Trumball, Ct., USA). The hollow fibre was filled with 10 μ l of 1-octanol. After 1 min, to ensure that the solvent would completely penetrate the pores, the a hollow fibre was immersed into the autosampler vials. The sample solution was stirred with a magnetic stir bar during extraction. After 30 min 1 μ l of octanol was withdrawn from the hollow fibre with a GC syringe and injected into the gas chromatograph. The gas chromatographic separation was achieved on a poly-(dimethylsiloxane) column (30 x 0.25 mm i.D., 0.25 mm film thickness) and the compounds were detected with a nitrogen-phosphorous detector (NPD). Helium (1 ml/min) was used a carrier gas. The chromatographic separation was achieved by temperature programming. The temperature was held at 180° C for 1 min and increased at 20° C/min to 300° C. Figure 5 shows chromatographs of the reference solution in octanol (5 nmol/ml) and chromatograms of the sample solutions (5 nmol/ml) of diazepam and prazepam in acetate buffer, in urine and plasma after enrichment by LLME. The chromatograms demonstrate preconcentration by a factor of 100 and 70, respectively, for diazepam and prazepam from the acetate buffer, urine and the plasma sample.

On page 12, please replace the last paragraph with the following paragraph:

LLME is performed with 1-octanol as the immobilized liquid. The A hollow fibre was immersed for 5 sec in 1-octanol which is sufficient for 1-octanol to penetrate and fill the pores of the fibre. 10 μ l of 0.1M HCl was used as acceptor solution and was filled into the impregnated fibre with a syringe. A standard solution of 4 μ g/ml of diphenhydramine in 0.1 M HCl was prepared as a reference for direct injection into the CE instrument. In addition, sample solutions of diphenhydramine (4 μ g/ml) were prepared in 0.1 M NaOH, in urine and plasma. Before extraction the pH in the urine and plasma sample solutions were adjusted to a pH 12-13 with NaOH. 1.5 ml of the sample solutions were placed in 2 ml autosampler vials. LLLME was accomplished by stirring with a magnetic stir bar for 30 min. The acceptor solution was removed after extraction and analysed by CE. Separations were performed inside a 10 cm effective length (52 cm total length) x 50 μ m internal diameter fused silica capillary. A 20 mM sodium acetate buffer adjusted to pH 4.5 with acetic acid was utilized as separation buffer. Sample introduction was accomplished by hydrodynamic injection with a pressure of 0.5 psi for 5 sec.

Separations were performed at 25 kV, while detection was accomplished at 215 nm. Electropherograms are shown in Figure 6. The electropherograms show that diphenhydramine (DH) was preconcentrated by a factor of 90 from the sample solution prepared in 0.1 M NaOH and in urine. A preconcentration of 50 was achieved from plasma. The lower enrichment from plasma is due to protein binding of the analyte. For both of the biological samples, excellent sample clean-up was observed in addition to analyte enrichment. In spite of the high sample complexity, almost no matrix components were observed in the electropherograms obtained by capillary zone electrophoresis.